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Distribution of Protein Kinase C-like Immunoreactive Neurons in Rat Brain

Naoaki Saito,[1] Ushio Kikkawa,[2] Yasutomi Nishizuka,[2] and Chikako Tanaka[1]

Departments of [1]Pharmacology and [2]Biochemistry, Kobe University School of Medicine, Kobe 650, Japan

Distribution of protein kinase C in the CNS of rat is presented based on immunohistochemical analysis with monoclonal antibodies against this protein kinase. Protein kinase C-like immunoreactivity was discretely localized and associated with neurons. Most, if not all, glial cells were not significantly stained. The greatest density of the immunoreactive material was seen in the following regions: the olfactory bulb (external plexiform layer), olfactory tuberculum, anterior olfactory nucleus, cerebral cortex (layers I and IV), pyriform cortex, hippocampus (strata radiatum and oriens), amygdaloid complex (central and basolateral nuclei), cerebellar cortex (molecular layer), dorsal cochlear nucleus, nucleus spinal tract of the trigeminal nerve, and dorsal horn of the spinal cord (substantia gelatinosa). Image analysis revealed that the regional distribution of the protein kinase C-like immunoreaction generally agreed with that of phorbol ester-binding sites. Immunoreactive perikarya were found in the following areas: the cerebral cortex (layers V and VI), caudate putamen, hippocampus, thalamus, amygdaloid complex, medial and lateral geniculate nucleus, superior colliculus, cerebellar cortex, nucleus spinal tract of the trigeminal nerve, dorsal cochlear nucleus, and dorsal horn of the spinal cord. Intense protein kinase C-like immunoreactivity in the neuron was observed both in the membrane and cytoplasm of the perikarya, dendrites, axons, and axon terminals, while weak immunoreaction was seen in the nuclei but almost never in the nucleoles. A map of protein kinase C-containing neurons was constructed. Such an uneven distribution in the brain suggests that this enzyme may play roles in controlling neuronal function in the areas noted.

Protein kinase C has been implicated as a common mechanism for the transduction of various extracellular signals into the cell to control many physiological processes (for reviews, see Nishizuka, 1984a, b, 1986). In the presence of both Ca^{2+} and phospholipids, this protein kinase is activated by 1,2-diacylglycerol, which transiently appears in the cell membrane as a consequence of receptor-mediated hydrolysis of inositol phospholipids (Takai et al., 1979; Kishimoto et al., 1980). Protein kinase C is known to be ubiquitously distributed in tissues. In particular, high amounts of this enzyme are present in the CNS (Kuo et al., 1980; Minakuchi et al., 1981; Kikkawa et al., 1982). Recent immunohistochemical analysis with polyclonal antisera against protein kinase C has shown that its intracellular distribution varies with cell type (Girard et al., 1985; Shoji et al., 1986; Wood et al., 1986). It has been clarified by complementary DNA analysis that multiple subspecies of this enzyme may exist in mammalian brain tissues (Coussens et al., 1986; Parker et al., 1986; Knopf et al., 1986; Makowske et al., 1986; Ono et al., 1986a, b; Ohno et al., 1987). More recently, an apparently homogeneous preparation of rat brain protein kinase C (Kikkawa et al., 1986) was shown to be further resolved into 3 distinct fractions, types I, II, and III, upon hydroxyapatite column chromatography (Huang et al., 1986; Kikkawa et al., 1987). The amino acid sequences of the 3 fractions were identified by comparison with the enzymes, which were separately expressed in COS cells transfected by the respective complementary DNAs (Kikkawa et al., 1987; Ono et al., 1987). In a preceding report (Kitano et al., 1987), 3 monoclonal antibodies—CKI-33, CKI-97, and CKII-90—were described that were raised against a mixture of the subspecies of rat brain protein kinase C. In the hope of clarifying the functional role of protein kinase C in the nervous system, we have made a preliminary survey of the distribution of this protein kinase in both brain and spinal cord of rats. Comparison of this enzyme pattern will also be made with that determined by phorbol ester binding.

Materials and Methods

Preparation of monoclonal antibodies. Protein kinase C was purified from the soluble fraction of the rat brain (Kikkawa et al.,1986), and 3 clones of monoclonal antibodies against the enzyme (CKI-33, CKI-97, and CKII-90) were prepared and the indetailed characteristics examined (Kitano et al., 1987). In the present studies, a mixture of the 3 antibodies was employed because the combined antibodies exhibited much stronger binding activity to protein kinase C than any of the antibodies alone, as described previously (Kitano et al., 1987).

Preparation of tissues. Wistar rats weighing 120–170 gm were anesthetized with pentobarbital (40 mg/kg, i.p.) and perfused through the left ventricle at a flow rate of 15 ml/min. The blood was removed with 30 ml of 0.9% NaCl at 4°C, and the brain was perfused at 4°C with 200 ml of a fixative containing 4% paraformaldehyde (FA), 0.2% picric acid (PA), and 0.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain was dissected, immersed for 48 hr in the postfixative containing 4% FA and 0.2% PA in PB at 4°C, washed with 30% sucrose in PB, and then cut on a cryostat or vibratome into frontal sections (20 μm). These sections were dipped directly in 0.1 M PBS containing 0.3% Triton X-100 (PBS-T) and were subsequently washed with the same buffer for at least 4 d at 4°C before use.

Immunohistochemical staining. The following steps were carried at 4°C unless otherwise indicated. The sections were incubated for 10 min with 0.3% H_2O_2 in PBS-T to inhibit the endogenous peroxidase and for 20 min with 3% normal goat serum (NGS) in PBS-T to block the nonspecific binding sites of proteins. The sections were washed with

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Correspondence should be addressed to Chikako Tanaka at the above address.

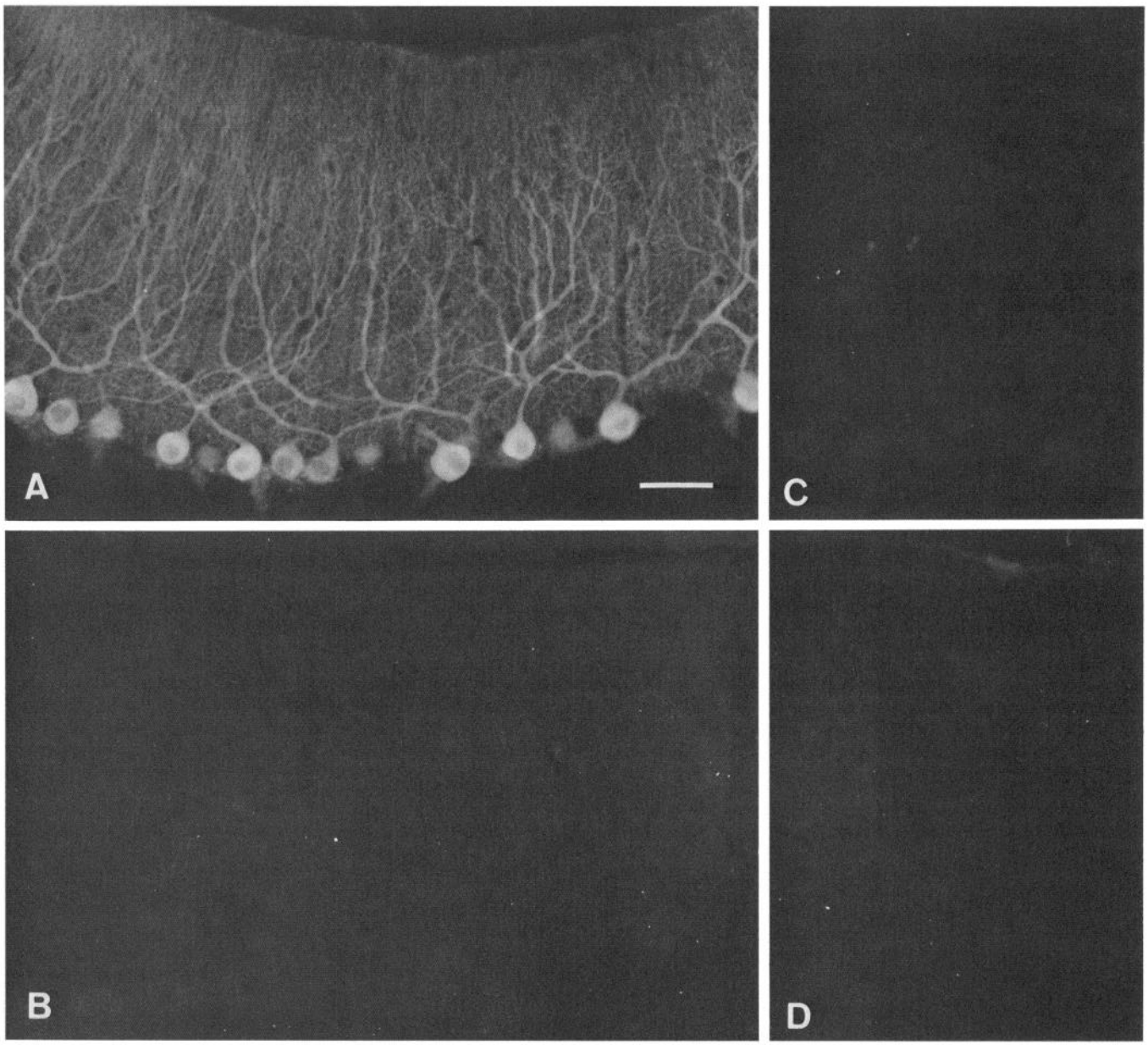

Figure 1. Absorption test of the monoclonal antibodies against protein kinase C in the immunohistochemical staining of sagittal sections of rat cerebellar cortex. ×25. Intense immunostaining of the Purkinje cells is seen in the section immunostained with nontreated mixed monoclonal antibodies (final concentration, 1 μg/ml) (*A*) but is completely abolished in the sections immunostained with preabsorbed monoclonal antibodies with purified C-kinase (5 μg/ml) (*B*), PBS containing Triton-X (0.1 M, pH 7.4) (*C*), normal mouse IgG (1 μg/ml) instead of monoclonal antibodies (*D*). Scale bar, 100 μm.

PBS-T and incubated with 3 monoclonal antibodies against protein kinase C (CKI-33, CKI-97, and CKII-90) in PBS-T for 18 hr to yield a final concentration of 1 μg/ml IgG. For peroxidase–antiperoxidase (PAP) immunostaining, the sections were washed again, incubated for 6 hr with goat anti-mouse IgG (Miles), diluted 1:1000, washed, and then incubated with mouse PAP complex (Miles) diluted 1:5000 for 1.5 hr. After rinsing 3 times, the preparations were reacted with 0.02% 3,3′-diaminobenzidine (DAB, Sigma) and 0.2% nickel ammonium sulfate in 50 mM Tris-HCl (pH 7.4) containing 0.005% H_2O_2. The preparations were dehydrated and coverslipped with Entellan (Merck) for light microscopic observation. For the immunofluorescent study, the sections were washed again and then incubated for 2 hr with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Miles) diluted 1:250. After rinsing 3 times, the sections were mounted in buffered glycerol for fluorescent microscopic observation.

Immunohistochemical control studies for the specificity were made by using PBS-T, normal mouse IgG, or the monoclonal antibodies which were preabsorbed with purified protein kinase C (5 μg/ml) instead of the monoclonal antibodies against protein kinase C.

^{3}H-PDBu autoradiography of phorbol ester binding sites. ^{3}H-phorbol 12,13-dibutyrate (PDBu) autoradiography was carried out by the method of Worley et al. (1986a). Briefly, Wistar rats weighing 120–170 gm were anesthetized, and the brain was perfused via the left ventricle with 50 mM sodium phosphate (pH 7.5)/0.3 M sucrose. The brain was dissected, cut on a cryostat at 7 μm, and mounted on gelatine-coated slides. The first section of 3 serial sections was fixed and immunostained with the monoclonal antibodies against protein kinase C by the PAP method described above. The second section was incubated with 2.5 nM ^{3}H-PDBu in 50 mM Tris-HCl buffer (pH 7.4) containing 100 mM NaCl and 1 mM $CaCl_2$. Nonspecific labeling was examined on the third section by adding 1 μM nonradioactive PDBu in the solution. After rinsing, the second and third sections were dried, attached to a Ultrofilm (LKB), and exposed for 7 d at 4°C. The autoradiogram and immunostaining were analyzed on an IBAS II image analyzer (Zeiss).

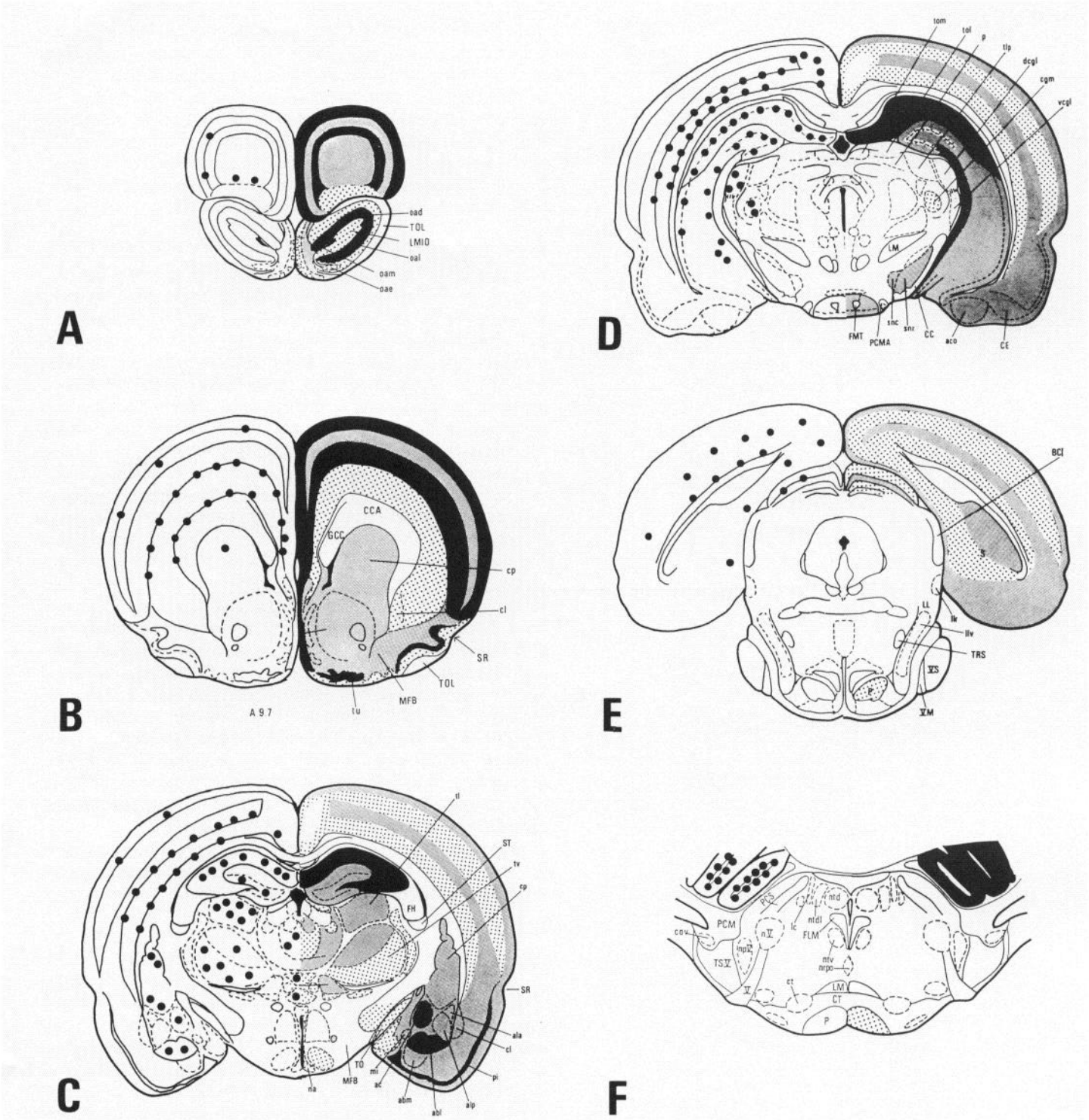

Figure 2. Atlases of the distribution of protein kinase C-like immunoreactivity in the frontal diagrams from forebrain to midbrain of the rat which are drawn based on the frontal sections stained by peroxidase–antiperoxidase immunohistochemistry. Total density of the immunoreaction (■, high; ▩, relatively high; ▨, relatively low; □, low) is indicated on the left-hand side. Protein kinase C-positive perikarya (type A) (●) are indicated on the right-hand side of the diagram (rostral; *A* to caudal; *F*).

Results

Control studies for immunohistochemical staining with monoclonal antibodies against protein kinase C

The monoclonal antibodies produced a selective staining pattern in the various sections of the rat brain. CKI-97 produced intense staining, but CKI-33 or CKII-90 alone produced poor staining. A mixture of the 3 antibodies, however, exhibited more intense positive staining than CKI-97 alone. In the cerebellar section, all Purkinje cells showed protein kinase C-like immunoreactivity, which was much greater in the cytoplasm of perikaryon and dendrite than in the nucleus and very low in the

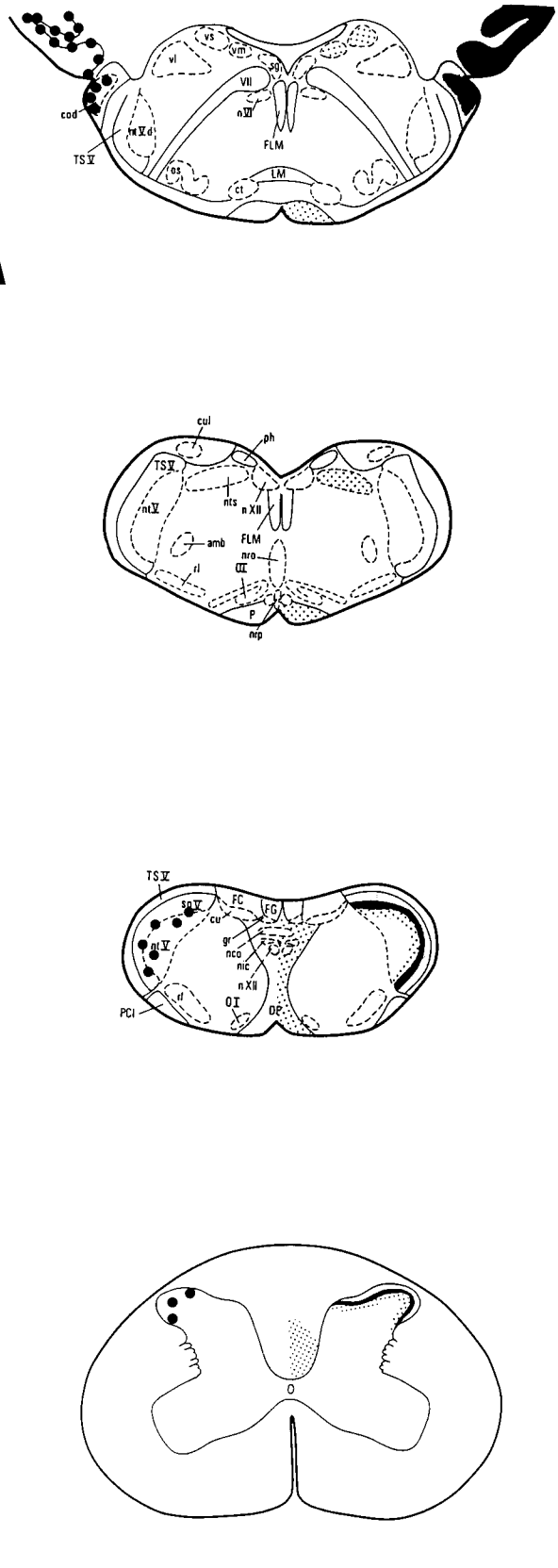

Figure 3. Atlases of the distribution of protein kinase C-like immunoreactivity in the frontal diagrams from hind brain to cervical spinal cord of the rat which are drawn based on the frontal sections stained by peroxidase–antiperoxidase immunohistochemistry. Total density of the immunoreaction (■, high; ■, relatively high; ▨, relatively low; □, low) is indicated on the left-hand side. Protein kinase C-positive perikarya (type A) (●) are indicated on the right-hand side of the diagram (rostral; *A* to caudal; *D*).

nucleolus (Fig. 1*A*). Preabsorption of the monoclonal antibodies with the purified protein kinase C (5 μg/ml), however, completely abolished the immunoreaction in the section of cerebellum (Fig. 1*B*). Similarly, no staining was observed in the cerebellar sections when PBS-T (Fig. 1*C*) or normal mouse IgG (Fig. 1*D*) was used instead of the monoclonal antibodies. By absorption tests, the immunostaining in other brain regions shown in Figures 5–11 was also identified as protein kinase C-like immunoreactivity (data not shown).

General distribution of protein kinase C-like immunoreactivity

The density of protein kinase C-like immunoreactivity was greatest in the following regions; the olfactory bulb (external plexiform layer), olfactory tuberculum, anterior olfactory nucleus, cerebral cortex (layers I and IV), pyriform cortex, hippocampus (strata radiatum and oriens), amygdaloid complex (central and basolateral nuclei), cerebellar cortex (molecular layer), dorsal cochlear nucleus, nucleus spinal tract of the trigeminal nerve, and dorsal horn of the spinal cord (substantia gelatinosa). Moderate densities of the protein kinase C-like immunoreaction were seen in the cerebral cortex (layers II, III, V, and VI), olfactory bulb (internal granular layer), caudate putamen, nucleus accumbens, medial forebrain bundle, dentate gyrus, thalamus (lateral, ventrolateral, and mediodorsal nuclei), amygdaloid complex (basomedial, anterolateral, centrolateral, and posteromedial nuclei), entorhinal cortex, substantia nigra, interpeduncular nucleus, deep cerebellar nuclei, and lateral vestibular nucleus. Moderately low densities of the immunoreaction were seen in the deepest layer of the frontal cortex, claustrum, thalamus (ventral and medial part of the medial nuclei), rhomboid nucleus, reunient nucleus, hypothalamus (arcuate and ventromedial nuclei), medial geniculate body, superior colliculus, pontine nucleus, vestibular nuclei (superior and medial nuclei), pyramidal tract, and solitary tract nucleus. The lowest immunoreactivity was present in the other regions of the lower brain stem and the white matter, such as the corpus callosum, capsula interna, and medial forebrain bundle.

The density of the protein kinase C-like immunoreactivity is illustrated in the series of frontal-sectional maps extending from the olfactory bulb to spinal cord (Figs. 2 and 3, right column).

Distribution of phorbol ester-binding sites

Phorbol ester-binding sites were studied in the sections adjacent to the immunostained sections. The distribution of ^{3}H-PDBu binding sites is shown in Figure 4 (left column) and compared with that of protein kinase C-like immunoreactivity (right column). High grain density of ^{3}H-PDBu binding and dense protein kinase C-like immunoreactivity were seen in the cerebral cortex, hippocampus, substantia nigra, and cerebellar cortex. In general, the distribution of ^{3}H-PDBu binding sites agreed with that of protein kinase C-like immunoreaction.

Distribution of perikarya containing protein kinase C-like immunoreactive material

Protein kinase C-like immunoreactivity characterized 2 types of perikarya.

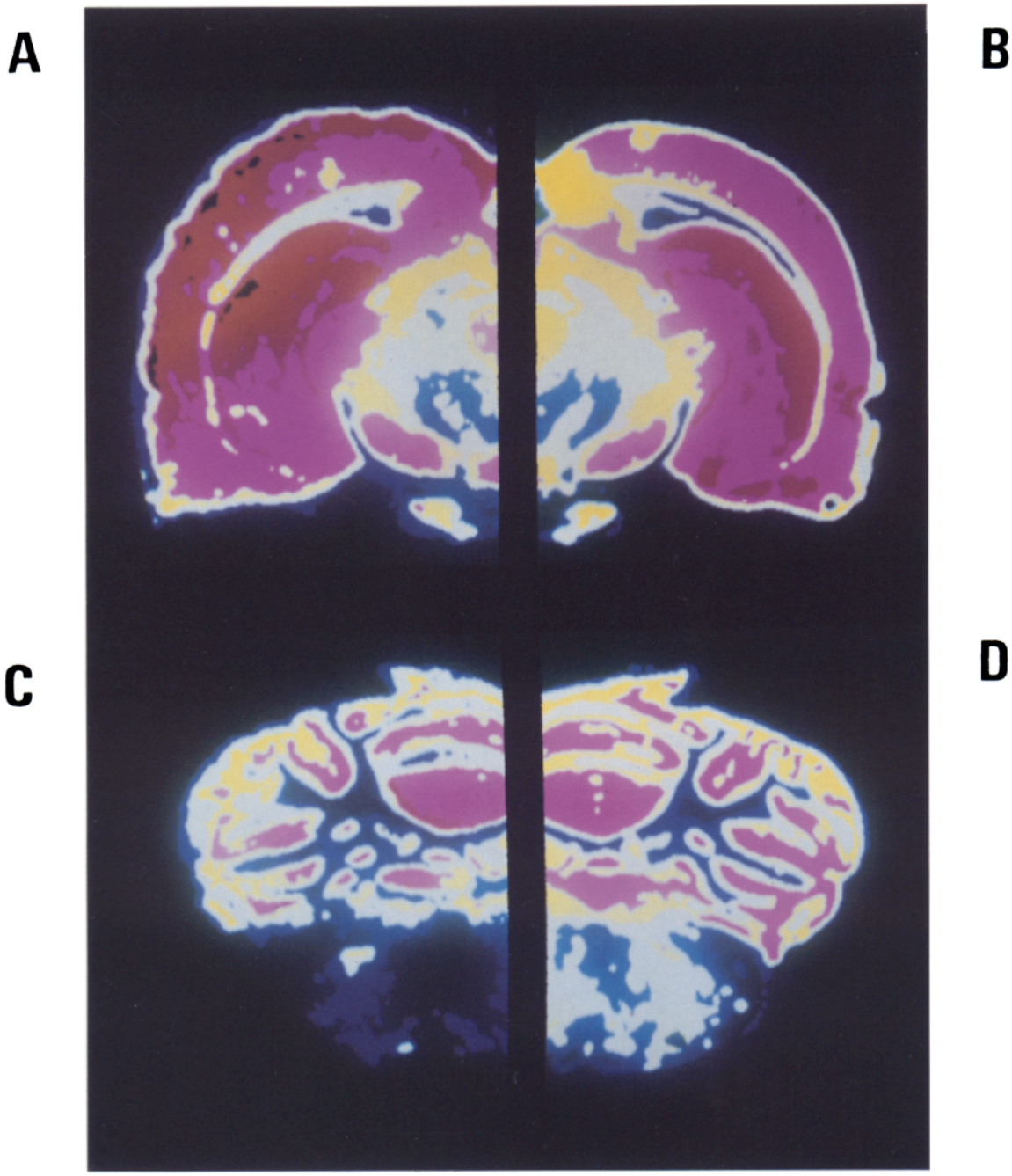

Figure 4. Comparison of the distribution of protein kinase C-like immunoreactivity and phorbol ester-binding sites in the frontal sections of the rat brain. Protein kinase C-like immunoreactivity was localized by peroxidase–antiperoxidase immunohistochemistry (*B, D*) and phorbol ester-binding sites are shown in the autoradiogram of ^{3}H-PDBu (phorbol 12,13-dibutyrate) binding (*A, C*). The color images of the autoradiogram roughly correspond to that of immunoreactivity in the section at the level of midbrain (*A, B*) and the cerebellum (*C, D*) but not of the lower brain stem (*bottom*). Relative density of both the autoradiogram and the immunoreactivity is shown according to the color table (highest density, red; lowest density, black).

Type A perikarya are characterized by the dense immunoreactivity in the cytoplasm. Some of these neurons have immunoreactive dendrites that were traced to some distance. The nucleus of these perikarya usually have poor immunoreactivity. This type of perikarya was seen in the following areas: cerebral cortex (layers IV and VI, particularly in the parietal cortex) (Fig. 5), caudate putamen (Fig. 6, *A, B*), hippocampus (pyramidal cells) (Fig. 6, *C, D*), amygdaloid complex (central and basolateral nuclei) (Fig. 7), medial and lateral geniculate nuclei, superior colliculus (superficial gray layer), cerebellar cortex (Purkinje cells; Fig. 8), dorsal cochlear nucleus (superficial layer) (Fig. 9*A*), thalamus (lateral, medial, and ventral nuclei) (Fig. 9, *B, C*), spinal tract of the trigeminal nerve, and dorsal horn of the spinal cord.

Type B perikarya have dense immunoreactivity on the perikaryal membrane but weak immunoreactivity in the cytoplasm. These perikarya were difficult to distinguish from unstained

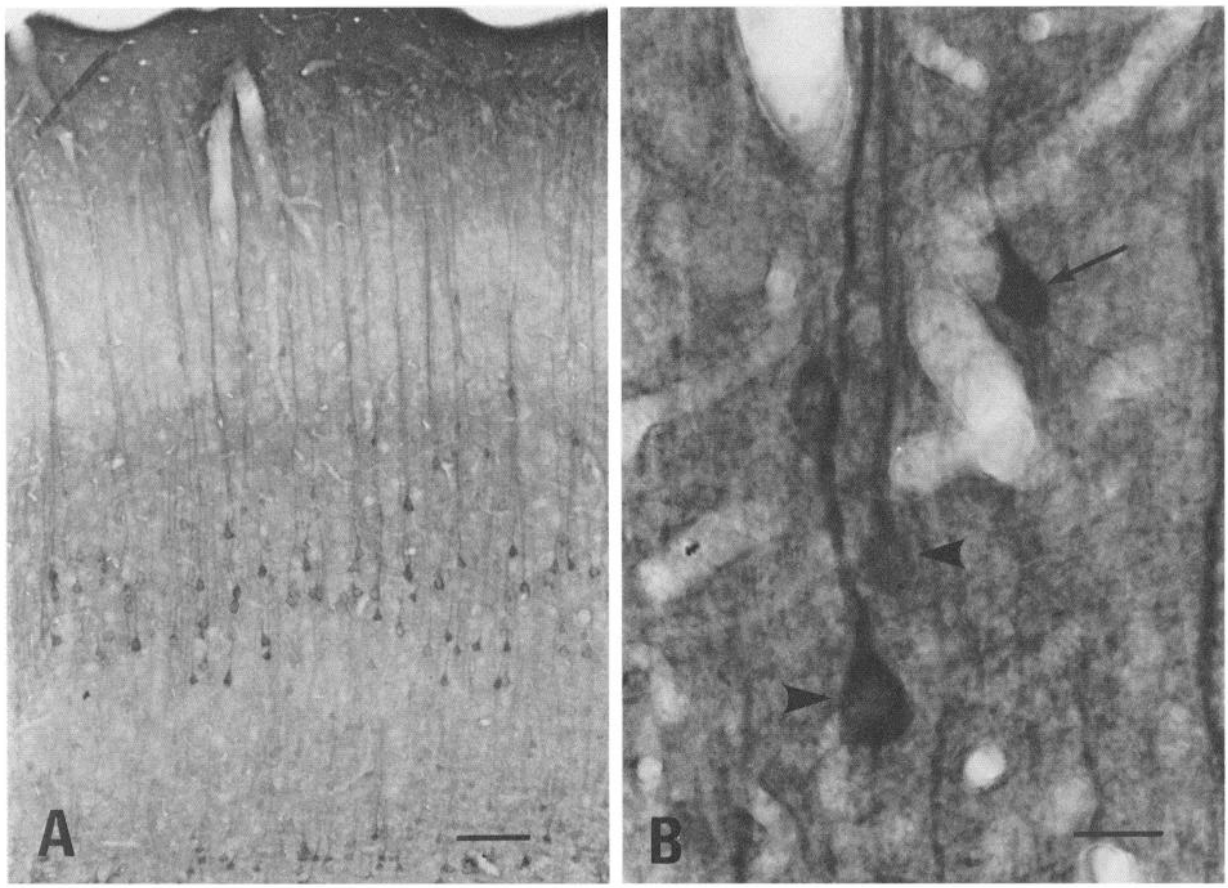

Figure 5. Protein kinase C-like immunoreactivity in frontal section of cerebral cortex as demonstrated by peroxidase–antiperoxidase immunohistochemistry. *A,* Dense immunoreaction in layers I, IV, and VI as demonstrated by the peroxidase–antiperoxidase technique. Pyramidal cells are seen with long apical dendrites extending vertically across layers IV–II. Scale bar, 100 μm. ×25. *B,* Immunoreactive pyramidal cells. Both perikarya and dendrites of the pyramidal cells (*arrowheads*) are strongly immunostained, but the nuclei are weakly stained. Multipolar medium-sized cell (*arrow*) is also seen in this layer. Scale bar, 20 μm. ×160.

perikarya located in the area densely packed with the immunoreactive neuropils. This type of perikarya was distinctly seen in the following areas: the pyriform cortex (Fig. 7), caudate putamen,and molecular layer of the cerebellar cortex (basket cells and stellate cells) (Fig. 8).

These 2 types of protein kinase C-like immunoreactive perikarya were distributed from rostral to caudal regions of the rat brain. The location of perikarya (type A) in the rat brain is presented in the series of frontal-sectional maps extending from olfactory bulb to spinal cord (Figs. 2 and 3, left column).

Intracellular localization of protein kinase C-like immunoreactivity

Protein kinase C-like immunoreactivity was found in various neuronal components, such as the perikaryal cytoplasm, dendritic cytoplasm, axons, axon terminals, and perikaryal membrane. Although the nuclei generally showed poor immunoreactivity, some immunoreactive nuclei were seen, whereas immunoreactive material in the nucleolus was not found. The immunostaining of the perikaryal cytoplasm was observed in the areas shown in Figures 1–3 and 5–11. In these regions, the immunostained dendritic cytoplasm was seen together with the immunostained perikaryal cytoplasm. The immunoreactive axon terminals were closely apposed to the cell surface of the unstained nerve cells in the deep cerebellar nuclei (Fig. 10, *A, B*), as well as in the lateral vestibular nucleus (Fig. 10, *C, D*). Stained axons were seen in the granular layer and white matter of the cerebellar cortex (Fig. 11), pyramidal tract (Figs. 2, 3), fornix (Fig. 2), diagonal bundle, anterior commissure (Fig. 2), and dorsal corticospinal tract (Fig. 3). The immunoreactive perikaryal membrane was clearly seen in the molecular layer of the cerebellar cortex (Fig. 8) and pyriform cortex (Fig. 7). However, immunoreactive perikaryal membrane was normally difficult to distinguish from the surrounding nerve terminals or dendrites.

Discussion

Morphological studies of the localization of protein kinase C have been performed by autoradiography using tumor-promoting phorbol esters (Nagle and Blumberg, 1983; Worley et al., 1986a), which directly activate protein kinase C (Castagna et al., 1982). The highest densities of this autoradiography were localized in the hippocampus, olfactory tubercle, neocortex (layer I), and cerebellar cortex, whereas the lowest density was in the white matter. Image analysis of autoradiogram and immunoreaction revealed that there are a number of similarities between the regional distribution of phorbol ester-binding and of protein kinase C-like immunoreactivity, indicating that the protein kinase C-like immunoreaction obtained here may represent most, if not all, of the enzyme in the rat brain.

More recent analysis of complementary DNA clones has clar-

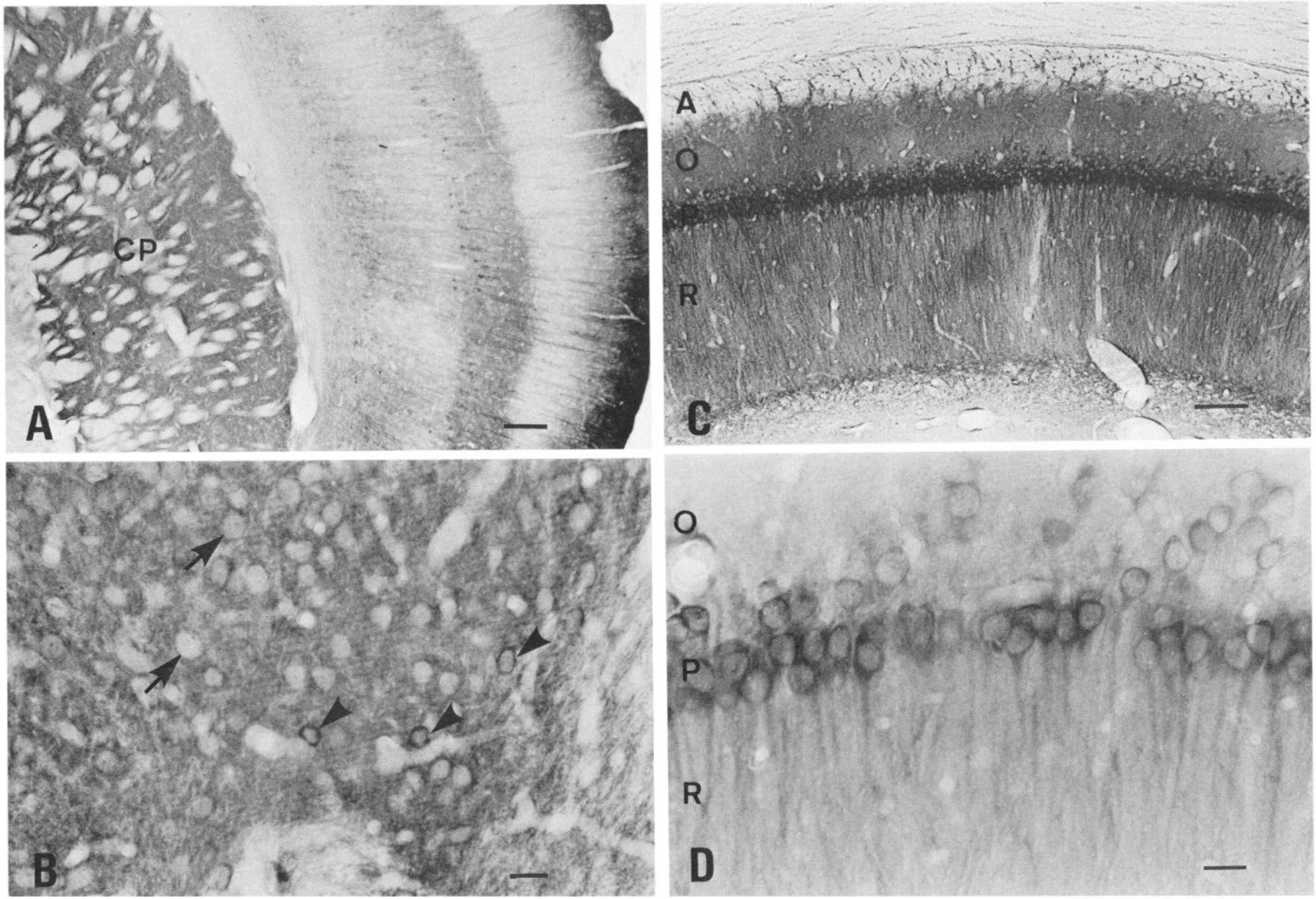

Figure 6. Protein kinase C-like immunoreactivity in frontal section of caudate putamen (*A* and *B*) and hippocampus (*C* and *D*) as demonstrated by peroxidase–antiperoxidase immunohistochemistry. *A,* Dense immunoreaction is present throughout the caudate putamen (*CP*) except the fiber bundles. Scale bar, 200 μm. ×10. *B,* Immunoreactive perikarya seen in the caudate putamen. A small number of scattered protein kinase C-immunoreactive perikarya (type A), small to medium-sized oval cells with nonimmunoreactive nuclei (*arrowheads*), are observed. Type B cells, which are larger cells with immunoreactive perikaryal membrane (*arrows*), are found in the CP. Scale bar, 20 μm. ×100. *C,* Immunoreactive perikarya of the pyramidal cells (*P*) of the hippocampus are seen. Both the stratum oriens (*O*) and stratum radiatum (*R*) show dense immunoreactivity. In the hippocampal alveus (*A*), nonimmunoreactive fiber bundles are seen to be encircled with immunoreactive fibers. Scale bar, 100 μm. ×25. *D,* Hippocampal pyramidal cells. Perikarya and dendrites of the pyramidal cells are heavily stained but the nuclei are not. Scale bar, 20 μm. ×100.

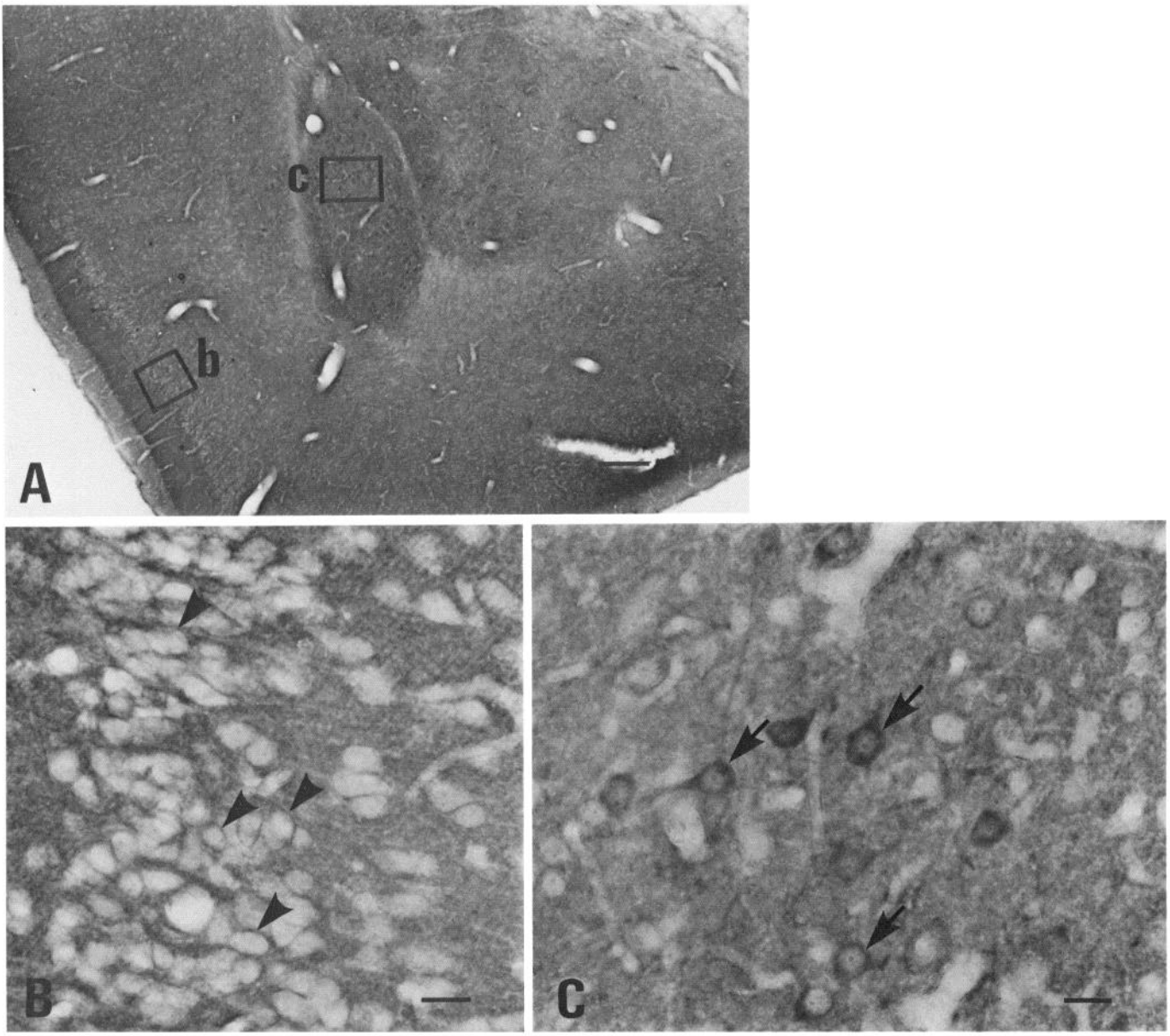

Figure 7. Protein kinase C-like immunoreactivity in frontal section of amygdaloid complex and pyriform cortex as demonstrated by peroxidase–antiperoxidase immunohistochemistry. *A,* Amygdaloid complex and pyriform cortex. Dense immunoreaction is seen throughout amygdaloid complex and pyriform cortex, the densest immunostaining being present in the basolateral and central nuclei of the amygdaloid complex. Frames indicate areas enlarged in *B* and *C.* Scale bar, 200 μm. ×10. *B,* Primary olfactory cortex. The medium-sized to larger cells in this area have immunoreactive perikaryal membrane (*arrowheads*). Scale bar, 20 μm. ×100. *C,* Basolateral nucleus of the amygdaloid complex. Immunoreactive perikarya of the medium-sized multipolar cell are seen (*arrows*). The long immunoreactive dendrite is traced to some distance. Scale bar, 20 μm. ×100.

ified the presence of multiple species of protein kinase C in the brain, as mentioned above. The nomenclature of α, βI, βII, and γ will be used hereafter for the 4 cDNA clones (Coussens et al., 1986; Kikkawa et al., 1987). We demonstrated that the fractions of types I–III are the subspecies of protein kinase C encoded by γ, β, and α-cDNA (Kikkawa et al., 1987).

Very recently, immunochemical analysis of these 3 antibodies has revealed that CKI-33 reacts weakly with enzyme types I–III, whereas CKI-97 reacts strongly and preferentially with type I, which corresponds to the enzyme encoded by γ- sequence, and CKII-90 reacts weakly with type II (unpublished observations). Theoretically, a mixture of the 3 antibodies used here can recognize all of the known subspecies of protein kinase C present in the rat brain. However, CKI-33 and CKII-90 provided poor immunostaining when used alone, while CKI-97 produced intense staining. Thus, the pattern with the 3 monoclonal antibodies was roughly the same as that obtained with CKI-97 alone, suggesting that type I enzyme may be preferentially visualized under the conditions used in the present studies. As will be described in detail in the following papers in this series, type I enzyme has thus far been found only in central nervous tissues.

Recent immunohistochemical studies by Kuo and coworkers, using polyclonal antisera against protein kinase C (Girard et al., 1985; Shoji et al., 1986; Wood et al., 1986), have demonstrated protein kinase C-like immunoreaction in the presynaptic ter-

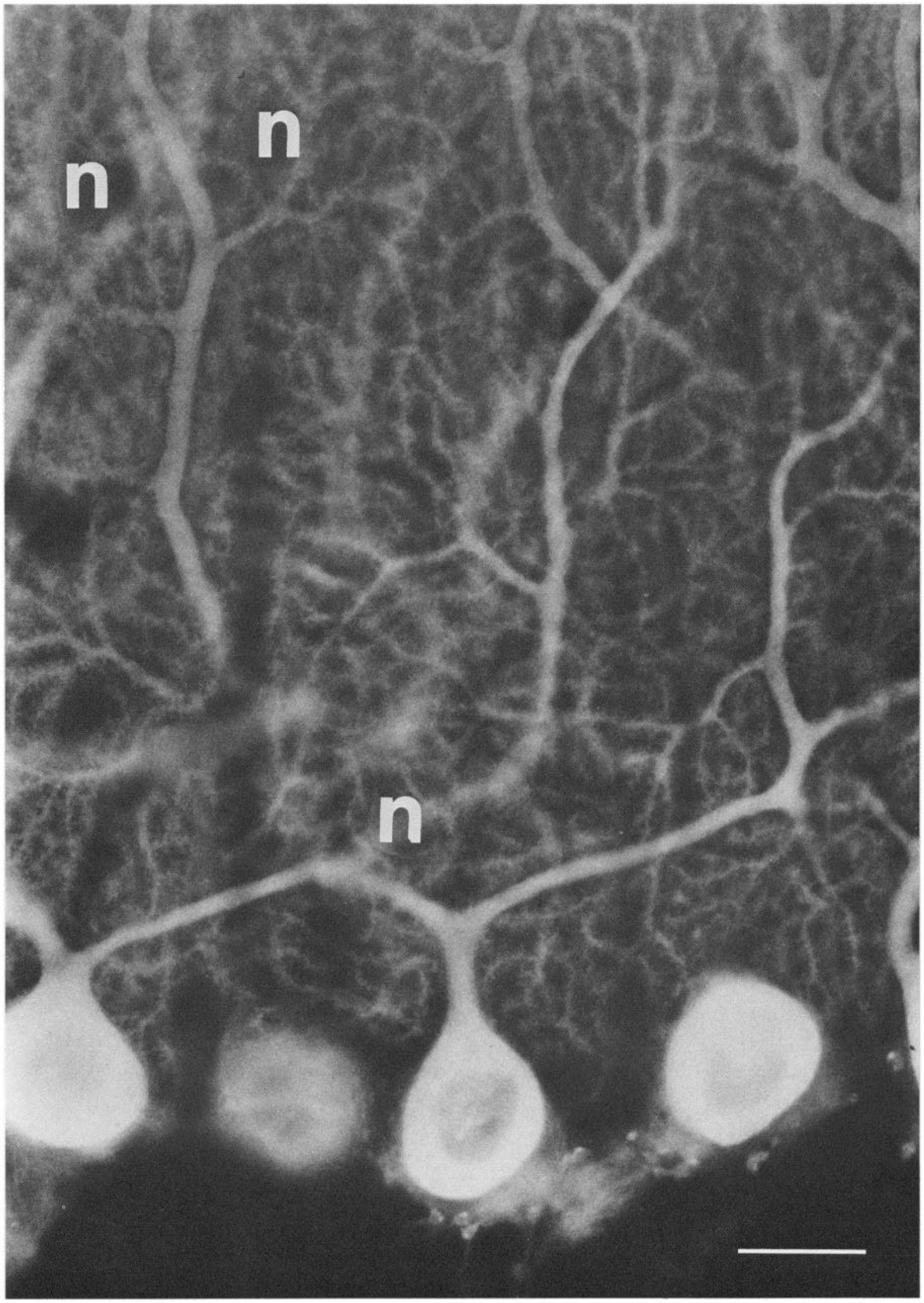

Figure 8. Protein kinase C-like immunoreactivity in sagittal section of cerebellar cortex as demonstrated by the indirect immunofluorescent technique. A unique feature of Purkinje cells is stained. The cytoplasm of all cellular components of Purkinje cells (perikaryon, dendrite, dendritic spine, axon) shows dense immunoreactivity and the nucleus shows weak immunoreactivity, while the nucleole does not. Among the network of dendrites, basket or stellate cells (*n*) are seen, which show dense immunoreactivity on their cell membranes. Scale bar, 50 μm. $\times 64$.

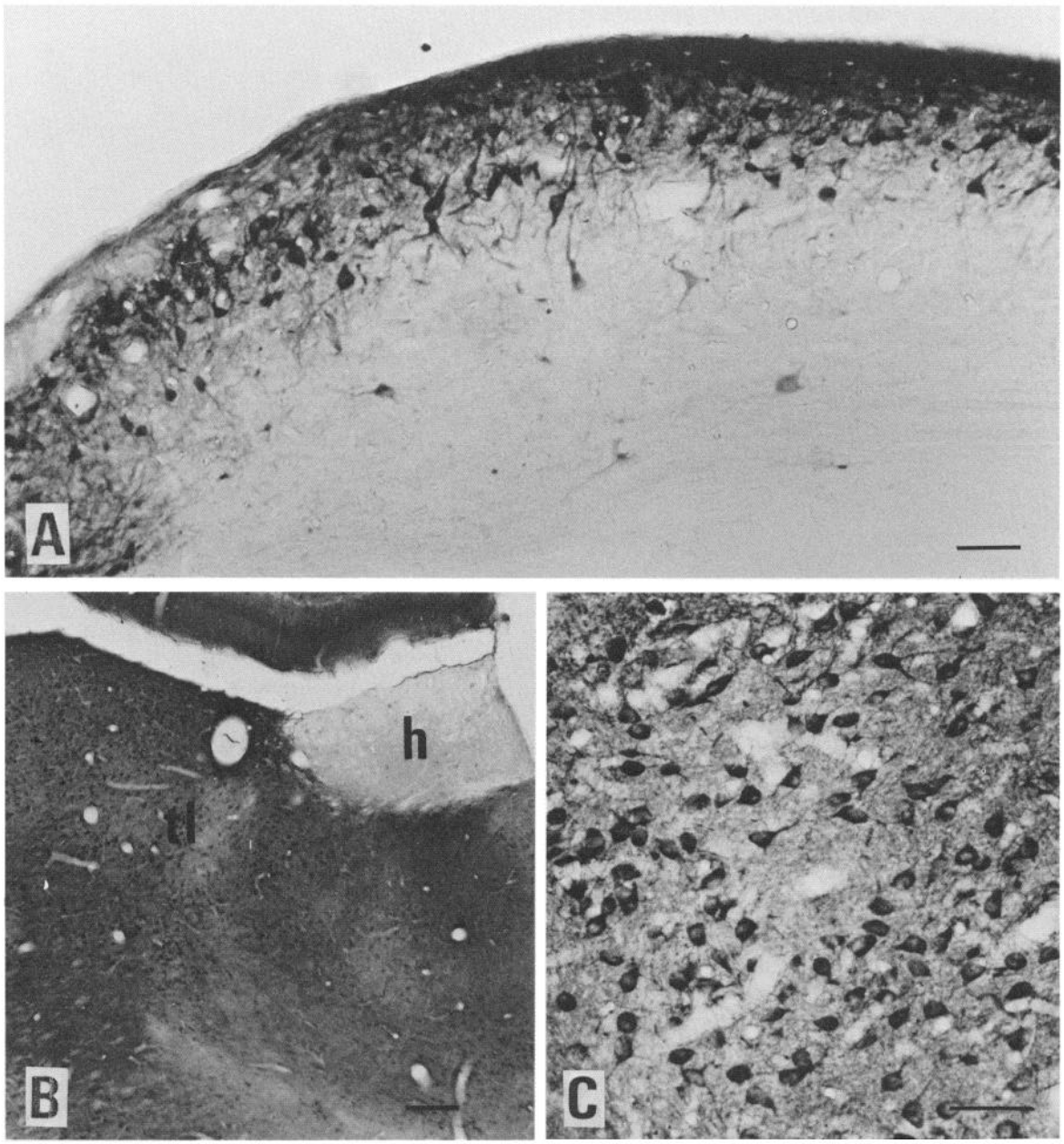

Figure 9. Protein kinase C-like immunoreactivity in frontal section of dorsal cochlear nucleus (*A*), habenular nucleus (*B*), and thalamus (*B, C*) as demonstrated by peroxidase–antiperoxidase immunohistochemistry. *A,* Immunoreactive neuropils are seen in the superficial layer of the dorsal cochlear nucleus. A considerable number of the stained multipolar or bipolar medium-sized cells are found in the superficial layer. Scale bar, 50 μm. ×25. *B,* Dorsal part of diencephalon. Immunoreactive perikarya are unevenly distributed in various thalamic nuclei. The prominent cluster of highly immunoreactive cells is found in the lateral nucleus (*tl*). No immunoreactivity is seen in the habenular nuclei (*h*). Scale bar, 200 μm. ×10. *C,* Lateral thalamic nucleus. The immunoreactive cells are found to be medium-sized spindle or oval cells with short dendrites. Scale bar, 50 μm. ×10.

minals, periphery of the nucleus of cerebral neurons, and oligodendroglia-like cells. These results appear to differ from those obtained in the present studies. The contradictions may result from differences in staining procedures and the characteristics of the antibodies employed. The polyclonal antisera used by Kuo's group were raised against pig brain protein kinase C and recognized 80 and 67 kDa proteins (Girard et al., 1985, 1986). On the other hand, the 3 monoclonal antibodies used here recognized only the 82 kDa protein in both purified and crude enzyme preparations (Kitano et al., 1987). Most recently, we have obtained antisera against oligopeptides of protein kinase C subspecies (βI and βII) that react with type II protein kinase C. Immunostaining with these antisera showed that the distribution of type II protein kinase C differs from that of type I in the brain and peripheral tissues of rats. The antisera used in Kuo's laboratory may recognize subtypes other than type I protein kinase C, which is predominantly stained by the monoclonal antibodies employed in the present studies.

Among various protein kinase C-positive neurons, a typical feature is seen in Purkinje cells of the cerebellar cortex, as reported previously (Kitano et al., 1987). A large amount of protein kinase C is present in somata as well as dendritic trees and

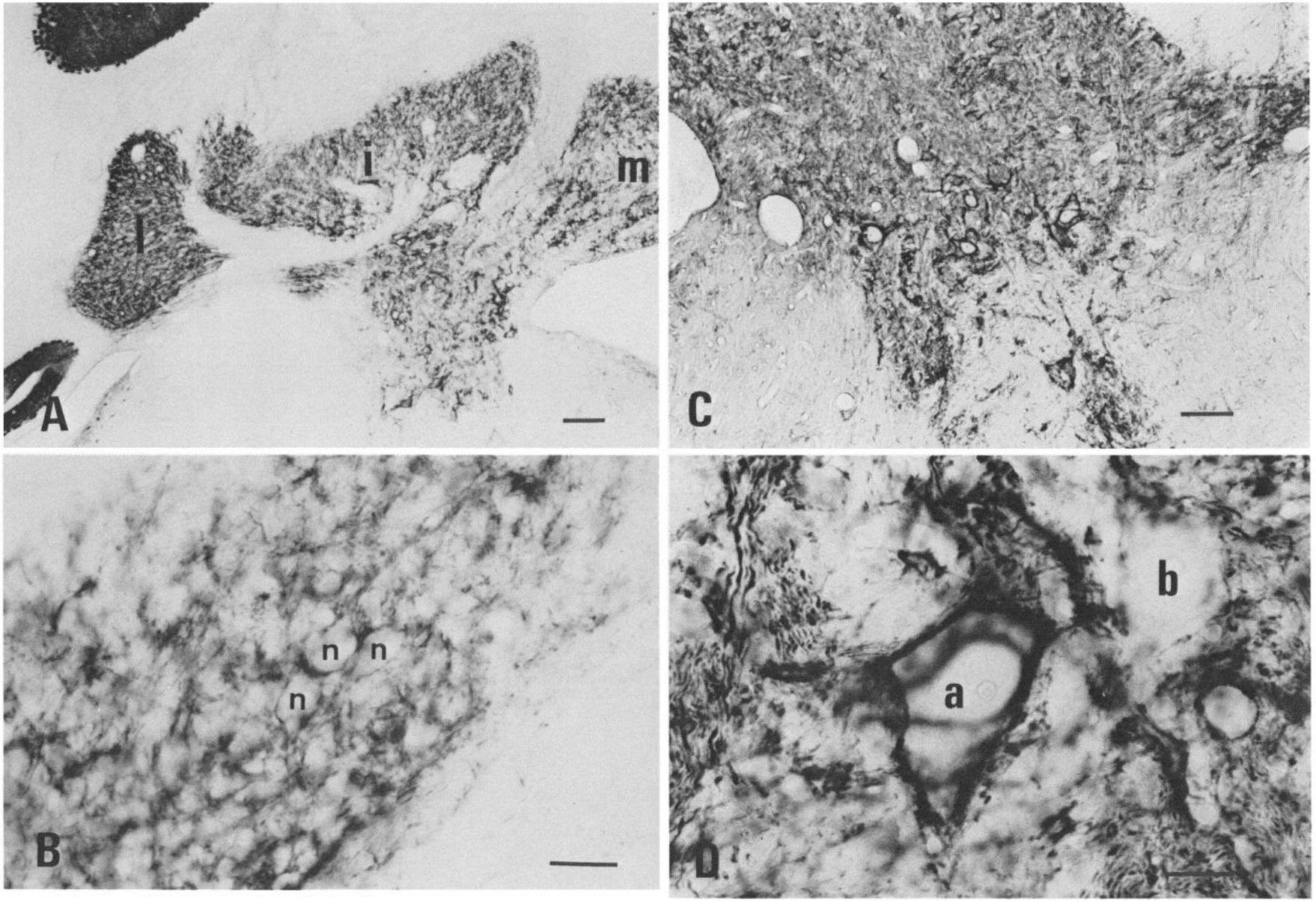

Figure 10. Protein kinase C-like immunoreactivity in frontal section of the deep cerebellar nuclei (*A* and *B*) and lateral vestibular nucleus (*C* and *D*) as demonstrated by peroxidase–antiperoxidase immunohistochemistry. *A,* Deep cerebellar nuclei. Moderately dense immunoreaction is seen in the lateral (*l*), interposutis (*i*), and medial (*m*) cerebellar nuclei. Scale, 200 μm. ×10. *B,* Interpositus cerebellar nucleus. Immunoreactive nerve fibers and nerve terminals are seen encircling large nonimmunoreactive perikarya (*n*) in the interpositus creebellar nucleus. Scale bar, 50 μm. ×16. *C,* Lateral vestibular nucleus. Among many immunoreactive fibers, nonimmunoreactive perikarya are seen to be surrounded with dense immunoreaction. Scale bar, 200 μm. ×25. *D,* Deiters cells in the lateral vestibular nucleus. The immunoreactive dots appear to represent the profiles of dendrites and perikarya of the Deiter's cell (*a*). Both Deiters cells (*a* and *b*) are unstained, but the left cell (*a*) is surrounded by many immunoreactive dots, while right cell (*b*) is not. Scale bar, 20 μm. ×160.

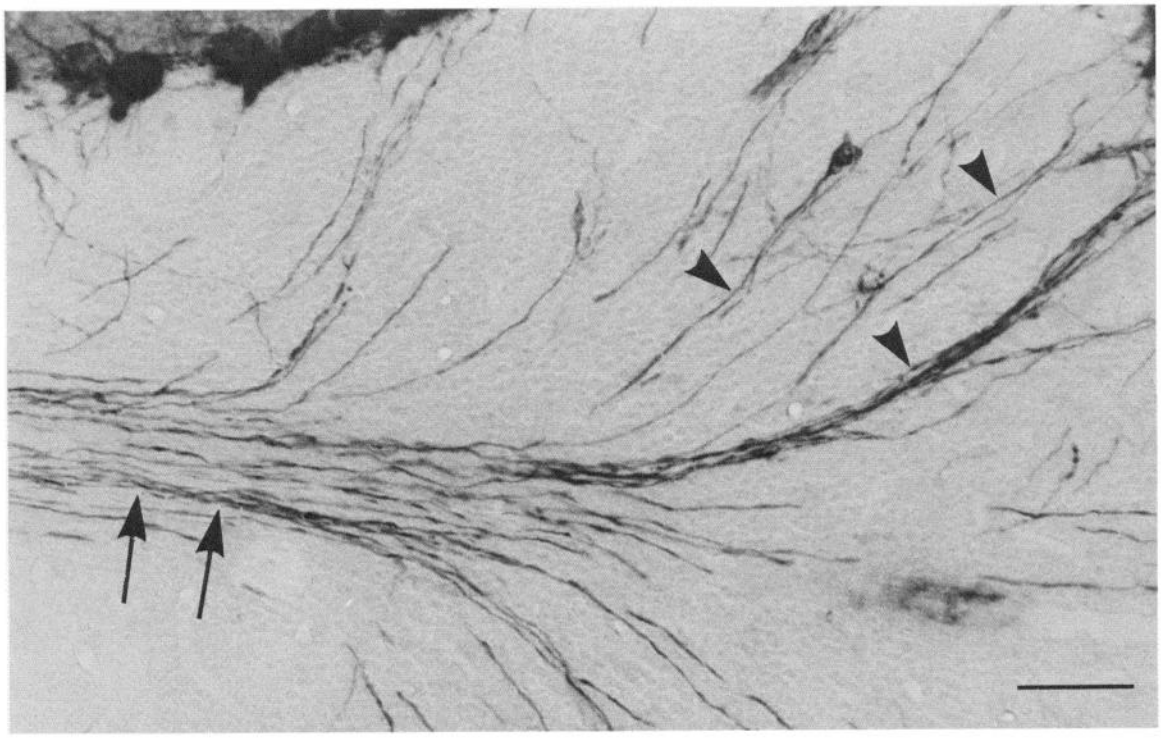

Figure 11. Protein kinase C-like immunoreactivity in a sagittal section of the granular layer of cerebellar cortex as demonstrated by peroxidase–antiperoxidase immunohistochemistry. Immunoreactive axons (*arrowheads*) of Purkinje cells are present in the granular layer with fine varicosities, while granule cells are not stained. The immunoreactive axons, seen running into the fiber bundle, run parallel with each other through the white matter (*arrows*). Scale bar, 50 μm.

many dendritic spines of Purkinje cells. The antiserum against cGMP-dependent protein kinase also stains Purkinje cells (De Camilli et al., 1984). The monoclonal antibodies against protein kinase C did not, however, react with cGMP-dependent protein kinase, and thus the present immunostaining does not appear to represent cGMP-dependent protein kinase (Kitano et al., 1987).

Kuo and coworkers (Girard et al., 1986; Wood et al., 1986) have also proposed that protein kinase C-like immunoreactivity is mainly associated with the presynaptic components, and not with perikaryal cytoplasm. In contrast, the present studies demonstrate protein kinase C-like immunoreactivity in many axon terminals and perikaryal cytoplasm, as cytoplasmic enzyme can be recognized by the monoclonal antibodies used under appropriate staining conditions. The involvement of protein kinase C in presynaptic neural functions has been proposed from the physiological findings that activation of protein kinase C enhances ACh release from ileal strips (Tanaka et al., 1984) and striatal slices (Tanaka et al., 1986), noradreneline release from the sinus node (Shuntoh and Tanaka, 1986), and dopamine release from cultured neurons (Zurgil and Zisapel, 1985). The present observations may provide a morphological basis for protein kinase C in the regulation of neurotransmitter release. Purkinje cells innervate the lateral vestibular nucleus and the deep cerebellar nuclei (Obata et al., 1967), and the immunoreactive terminals are attached to Deiter's cells in the lateral vestibular nucleus and also to the neurons of the deep cerebellar nuclei. Immunoreactive nerve terminals are also seen in the neuropils of the substantia nigra and caudate putamen.

In the hippocampus, protein kinase C has been suggested to be associated with the generation of long-term potentiation (LTP), as LTP elevates the phosphorylation of protein F1, which is a substrate of protein kinase C (Akers et al., 1985, 1986; Malenka et al., 1986). The present results reveal that a large amount of protein kinase C is contained in the hippocampal cortex, especially in perikarya and apical dendrites of the pyramidal cell, which is a postsynaptic component of the site where LTP has been thoroughly characterized. This suggests that protein kinase C is involved in the generation of LTP in the postsynaptic component, in addition to presynaptic control of transmitter release in the hippocampus (Malenka et al., 1986).

The distribution of protein kinase C-like immunoreactivity does not always correspond to that of any of the classical neurotransmitters such as monoamines, ACh, amino acids, and neuropeptides. It also differs from that of other second-messenger systems [adenylate cyclase, guanine nucleotide-binding protein (G_0)] (Worley et al., 1986b, c). There are some differences between the localization of guanine nucleotide-binding protein and that of protein kinase C-like immunoreactivity. For example, pyramidal cells in the hippocampus are not stained with antiserum against guanine nucleotide-binding protein; protein kinase C, however, is present in the pyramidal cells, where the immunoreaction is seen in the thin cytoplasm but not in the large nucleus. These observations suggest that protein kinase C is not always associated with guanine nucleotide-binding protein.

Worley et al. (1987) have also observed that the distribution of radioactive inositol 1,4,5-trisphosphate (IP_3) binding is similar to that of protein kinase C in the cerebral and cerebellar cortices but not in the substantia gelatinosa of spinal cord. We suggest that the IP_3 branch may play a prominent role in the cerebellum, while in the substantia gelatinosa of spinal cord, the

action of protein kinase C may predominate. However, intense protein kinase C-like immunoreactivity was observed in the dendritic spines of Purkinje cells, which possess a subsurface specialization of the endoplasmic reticulum, an intracellular source of calcium (Henkart et al., 1976; Fifkova et al., 1983); this suggests prominent roles for protein kinase C and IP_3 in the cerebellum.

In conclusion, the present studies may provide morphological evidence for the possible involvement of protein kinase C in both presynaptic and postsynaptic functions. It must be recognized, however, that the presence of immunonegative areas or immunonegative subcellular components, such as the corpus callosum or nucleus, does not reflect the absence of protein kinase C, especially of other types of this enzyme family.

Appendix

Abbreviations used in the figures

abl, nucleus amygdaloideus basalis, pars lateralis
abm, nucleus amygdaloideus basalis, pars medialis
ac, nucleus amygdaloideus centralis
aco, nucleus amygdaloideus corticalis
ala, nucleus amygdaloideus lateralis, pars anterior
alp, nucleus amygdaloideus lateralis, pars posterior
amb, nucleus ambiguus
BCI, brachium colliculi inferioris
CC, crus cerebri
CCA, corpus callosum
CE, cortex entorhinalis
cgm, nucleus centralis corporis geniculati medialis
cl, claustrum
cod, nucleus cochlearis dorsalis
cov, nucleus cochlearis ventralis
cp, nucleus caudate putamen
ct, nucleus corporis trapezoidei
cu, nucleus cuneatus
cul, nucleus cuneatus lateralis
dcgl, nucleus dorsalis corporis geniculati lateralis
FC, fasciculus cuneatus
FH, fimbria hippocampi
FLM, fasciculus longitudinalis medialis
FMT, fasciculus mamillothalamicus
GCC, genu corporis callosi
gr, nucleus gracilis
lc, locus coeruleus
LL, lemniscus lateralis
llr, nucleus lemnisci lateralis rostralis
llv, nucleus lemnisci lateralis ventralis
LM, lemniscus medialis
LMIO, lamina medullaris interna bulbi olfactorii
mi, massae intercalatae
na, nucleus arcuatus
nco, nucleus commissuralis
nic, nucleus intercalatus
npV, nucleus sensorius principalis nervi trigemini
nro, nucleus raphe obscurus
nrp, nucleus reticularis paramedianus
nrpo, nucleus raphe pontis
ntd, nucleus tegmenti dorsalis
ntdl, nucleus tegmenti dorsalis lateralis
nts, nucleus tractus solitarii
ntv, nucleus tegmenti ventralis
ntV, nucleus tractus spinalis nervi trigemini
ntVd, nucleus tractus spinalis nervi trigemini, pars dorsomedialis
nV, nucleus originis nervi trigemini
nVI, nucleus originis nervi abducentis
nXII, nucleus originis nervi hypoglossi
oad, nucleus olfactorius anterior, pars dorsalis
oae, nucleus olfactorius anterior, pars externa
oal, nucleus olfactorius anterior, pars lateralis
oam, nucleus olfactorius anterior, pars medialis
OI, oliva inferior
os, nucleus olivaris superior
p, nucleus pretectalis
P, tractus corticospinalis
PCMA, pedunculus corporis mamillaris
PCS, pedunculus cerebellaris medius
ph, nucleus prepositus hypoglossi
rl, nucleus reticularis lateralis
S, subiculum
sg, nucleus suprageniculatus facialis
sgV, nucleus tractus spinalis nervi trigemini, substantia gelatinosa
snc, substantia nigra, pars compacta
snr, substantia nigra, pars reticularis
ST, stria terminalis
TO, tractus opticus
tol, nucleus tractus optici, pars lateralis
TOL, tractus olfactorius lateralis
tom, nucleus tractus optici, pars medialis
TRS, tractus rubrospinalis
TSV, tractus spinalis nervi trigemini
tu, tuberculum olfactorium
tv, nucleus ventralis
vcgl, nucleus ventralis corporis geniculati lateralis
vl, nucleus vestibularis lateralis
vm, nucleus vestibularis medialis
vs, nucleus vestibularis superior
VII, nervus facialis
VM, nervus trigeminus, radix motoria
VS, nervus trigeminus, radix sensoria

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